2D MAPPING OF STRONGLY DEFORMABLE CELL NUCLEI BASED ON CONTOUR MATCHING

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Abstract

The spatiotemporal dynamics of protein complexes and genome loci are functionally linked to cellular health status. In order to study the inherent motion of subnuclear particles, it is essential to remove any superimposed component stemming from displacement and deformation of the nucleus. Several models have been proposed to cope with transformations occurring during basal pan-nuclear dynamics of healthy cells. However, in pathological cells, such as lamin A deficient fibroblasts, the nucleus deforms in a dramatic and apparently random way, making the use of such models a non trivial task. In this paper we propose a mapping of the nuclear interior, which is based on the deformation of the nucleus. The proposed method does not put constraints on the possible shapes or deformations. The primary goal of this registration procedure is to enable an accurate estimation of telomere mobility in living human cells undergoing dramatic nuclear deformations.

Key Terms

Image Registration, Contour Matching, Shape Normalization, Nuclear dynamics

Introduction

The internal organisation of the human cell nucleus in space and time is essential for its function. Within the limited space of the nucleus the entire genome as well as many proteins are accommodated in a non-random manner. Of particular interest are telomeres, the very ends of chromosomes, which show a spatiotemporal behaviour that is functionally linked to cellular and organismal condition. Telomeres are arranged in distinct patterns and display mobility regimes at different time scales (1,2). Diverse biological processes such as telomere maintenance, senescence and apoptosis are associated with characteristic telomere redistributions (3,4). Likewise, altered telomere dynamics are associated with specific diseases such as laminopathies and cancer (5,6). Therefore, quantitative studies of telomere dynamics – and by expansion nuclear dynamics - may help reveal novel mechanisms of dysfunction and disease. However, mobility analyses in four-dimensional image data sets are seriously hampered by global cell motion and nuclear deformation. This superimposes a motion on the submicron dynamics, which in turn, creates a significant bias on the accuracy of the measurements. It is therefore imperative to remove the superfluous motion by means of image registration. A wide range of registration techniques has been proposed to remove 2D and 3D motion of cell nuclei using rigid transformations, i.e. translation and rotation (7-9). However, human cells often display global and local expansions or contractions, which cannot be adequately modelled by rigid transformations. To cover for this biological variation, a mapping based on a spherical model has been proposed (10), which captures most of the cellular and nuclear displacements that occur in normal cells during interphase. However, under certain pathological conditions, such as cancer and laminopathies, the nuclear shape alters dramatically (11), creating a situation in which a spherical model can no longer be applied (Suppl. Movie S1). Hence, a method is required that allows reliable motion measurements of subnuclear features in a deformable volume without imposing any shape

constraints. To cater for complete non-rigid transformations, landmark-based methods have been proposed (12,13), but the results of these methods are completely dependent on the choice of robust landmarks, which is not always straightforward.

In this paper, we propose a novel approach, which maps two nuclei between consecutive time points without imposing constraints (e.g. shape), nor requiring any landmarks. Instead, contours are extracted from segmented nuclei, which are matched in order to achieve a point-to-point correspondence. Based on this matching, a mapping of the full nucleus is calculated using polyharmonic splines. This mapping predicts the location of nuclear loci, solely based on the deformation of the nucleus, i.e. in the absence of individual motion. The actual displacements can then be determined by comparing the predicted location with the actual location of the nuclear loci of interest. The algorithm was tested both on simulated images and on recordings of human cells undergoing artificially induced as well as naturally occurring deformation. Using this algorithm, the overall accuracy was improved significantly compared to rigid registration or state of the art Demons method (14). In addition, the algorithm significantly increased the tracking efficiency by dramatically reducing the number of false matches in the nearest neighbour based tracking algorithm.

Materials and Methods

Cell culture and transfection

ECV-TRF2 were cultured in advanced DMEM (Gibco, Invitrogen, USA) supplemented with 2% FCS and 1% pencillin/streptomycin/glutamin. ECV-TRF2, is a derivative clone from ECV304 – a cell line originally described as an immortalized endothelial cell line (15) – stably expressing the telomere binding fusion protein mCitrine-hTRF2 (16). Fibroblast cells from a patient with a nonsense Y259X homozygous mutation in lamin A/C gene ($LMNA^{-/-}$) which are completely devoid of functional lamin A/C (17), were cultured in advanced DMEM/F12 (Gibco, Invitrogen, Merelbeke, Belgium) supplemented with 10% FCS and 1% penicillin/streptomycin/glutamin.

For transfections and imaging, cells were grown on collagen coated glass bottom dishes (Matek, USA). $LMNA^{-/-}$ cells were transiently transfected with the construct pmCitrine-hTRF2 and Lipofectamin 2000 (Invitrogen, Merelbeke, Belgium) according to manufacturers' instructions. Transiently transfected cells were imaged 24h after transfection.

Image acquisition

For live cell imaging, glass bottom dishes were filled with pre-warmed medium and sealed off with parafilm, after which they were inserted into an incubator microscope and allowed to equilibrate to 37°C for 30 minutes. Cells were observed under a Nikon A1r confocal microscope mounted on a Nikon Ti body (Nikon Instruments, France). Experiments were carried out with a Nikon Plan Apo 40x/1.3 objective. Two-hour time-lapse recordings of $LMNA^{-/-}$ cells were made at 2 min intervals with minimally invasive CLEM technology (18). For the validation experiments, ECV-TRF2 cells were chemically fixed by addition of cold (4°C) 100% methanol (Sigma, Belgium) in fluxo, i.e. during acquisition at 30 sec intervals.

Image Processing

We have developed a novel method for full non-rigid registration of nuclei in raw CLEM images. All processing work was performed in Matlab R20007b® on an Intel i7 1.6GHz processor with 4 GB memory. A workflow was created of which the major aspects are described in more detail below and which are schematically summarized in Fig. 1.

I. Segmentation

Since the proposed method estimates the topological changes based on the deformation of the nucleus, the nucleus needs to be segmented first (Fig. S1). To this end, an average projection is made of the confocal 3D stack, followed by a low pass filter to reduce noise. The resulting image is then thresholded using Otsu's thresholding method, which calculates a threshold by minimizing the variance of both the foreground and background (19). Due to remaining noise, not all nucleus pixels are correctly classified as foreground; also some background pixels are wrongfully considered to be foreground. This is corrected with a morphological opening and closing. Finally the contour delineating the segment is parameterised and filtered with a Gaussian kernel, in order to obtain a smooth contour. This is important for the subsequent signature matching (see next paragraph). For more detailed work on cell nuclei segmentation, we refer to (20-22).

II. Contour Matching

Next, we find the correspondence between contours delineating the nucleus in consecutive time frames: points belonging to the contour in one frame will be linked to the corresponding points on the contour in the next frame. In order to do so, we first calculate a signature of the contour. This is a function describing the contour in one dimension. The signature is then used for the actual matching. The contour is described as $\boldsymbol{c}(t) = (x(t), y(t))$, with $t \in \mathrm{Z}$ and

$\mathbf{c}(N+1) = \mathbf{c}(1)$, i.e. the contour is periodical. Four different signatures were calculated based on $\boldsymbol{c}(t)$: a complex signature, a normalized centroid distance signature, a tangent direction signature and a curvature signature (Fig. S2). For a more detailed description of these signatures, see the appendix. The matching criterion we applied for two signatures is the Dynamic Time Warping (DTW) or dog-man distance. The DTW distance is the smallest possible cumulative difference between both signatures, which is found by changing the scanning speed along the contour (signature) (Fig. 1c), i.e.

$$d_{DTW}(s_1(.), s_2(.)) = \min_\gamma \sum_t \|s_1(t) - s_2(\gamma(t))\| \quad (1),$$

where $\gamma(.)$ is a warping function, i.e. a monotonic function mapping of $[1, N]$ onto $[1, N]$. For contours defined on a discrete grid, this can be calculated in an efficient way using dynamic programming:

$$D(i,j) = d(i,j) + \min(\alpha D(i,j-1), \alpha D(i-1,j), D(i-1,j-1)) \quad (2)$$

With $d(i,j) = \|s_1(i) - s_2(j)\|$ and α a real number greater than or equal to one. In order to penalize warping functions where too many points of one signal are matched to a single point in the other signal, one can set $\alpha > 1$, for $\alpha = 1$, all warping functions are considered equally good. For the purpose of matching nuclei contours in our datasets, α was set to 1.25. The DTW distance is uniquely defined between two finite time series. On the other hand, the signatures are infinitely long, be it periodical, which makes that the signature between 0 and N can be considered finite in time. This, however, assumes that the starting point of the contour corresponds to the starting point in the other contour. If this is not the case, a wrong DTW distance and a wrong optimal warping function will be obtained. This potential pitfall is avoided by calculating the DTW distance twice: once to find the offset and a second time to find exact matching. The first time the DTW is calculated, generally results in a warping function where the start point of a signal is matched to multiple points on the other signal (Fig. 1d). If the starting point is matched to k different points, the signal is circular shifted k

places such that the starting point corresponds to exactly a single point. The DTW distance is now calculated for a second time, but now between the first signal and the shifted version of the second signal. The DTW distance itself is of little importance, but the warping function, $\gamma(.)$ resulting in the minimal distance, is. This warping function gives the correspondence between points from both signatures. It may happen that multiple points on one contour match a single point on the second contour. This might lead to unstable solutions in the cell-mapping algorithm (see next subsection). To avoid this situation both contours are resampled. Consider the case, where one point, $s(t)$ of the signature is matched with multiple points on the other signature (cfr. Fig. 1e). First define $s'(t-1)$ and $s'(t+1)$ as the last predecessor resp. the first successor of $s(t)$ with a unique point-to-point correspondence, e.g. $s'(t-1)$and $s'(t+1)$ respectively. The curve between $s'(t-1)$ and $s'(t+1)$ will be resampled in such a way that there are as many samples as there are samples between $s(t-1)$ and $s(t+1)$, where the samples are calculated such that the arclength between the samples is proportional to the arclength of their corresponding samples on the other signal (i.e. time point). In the example shown in Fig. 1e, this results in the replacement of $s(t)$ by two new samples, shown as black dots in the figure.

III. Cell Mapping

Based on the contour matching, each point on the contour will now correspond to a unique point on the contour of the subsequent frame. For mapping of points inside of the nucleus, $\mathbf{p} = (x, y)$, we implement polyharmonic spline interpolation:

$$M(\mathbf{p}) = a + a_x x + a_y y + \sum_{j=1}^{N} \mathbf{w}_j \phi(d(\mathbf{p} - \mathbf{c}(j)) \qquad (3)$$

where a, a_x, a_y and $\mathbf{w}_j$ are a set of weighting coefficients, $\mathbf{c}(j)$ are the contour points, d is a distance function and $\phi(.)$ is a radial basis function. We propose the commonly used

polyharmonic splines as radial basis functions, i.e.

$$\phi(r) = \begin{cases} r^k, & if\ k\ is\ odd \\ r^k \ln r, & if\ k\ is\ even \end{cases} \qquad (4),$$

where k is the degree of the basis functions. The weighting coefficients in eq. (3) are calculated by solving a system of linear equations:

- N equations that map the points on the contour to their matching points on the other contour, i .e. $M(\mathbf{c}(\mathrm{t})) = \mathbf{c}'(\mathrm{t})$ where $\mathbf{c}'(\mathrm{t})$ is the matching contour point of $\mathbf{c}(\mathrm{t})$.
- two equations imposing orthogonality of the weighting coefficients, $\mathbf{w}$ onto the basis function, i.e. $\sum_{i=1}^{N} \mathbf{w}_i x_i = \sum_{i=1}^{N} \mathbf{w}_i y_i = 0$.
- one equation forcing the weighting coefficients to sum to zero: $\sum_{i=1}^{N} \mathbf{w}_i = 0$

This system can be solved using any solver for linear systems, e.g. Gaussian elimination. For a fast solver for this specific linear system we refer to (25). A more detailed discussion on polyharmonic splines and the influence of the different radial basis functions can be found in (26).

Although polyharmonic interpolation has been proven useful for non-rigid registration, some adjustments are needed in order to use them in this context. The mapping of a point in the nucleus depends on the Euclidean distance between the point itself and the contour points. This distance however does not take the nuclear shape into account. By ignoring the nuclear shape we overestimate the motion of contour points which might be close by in the image, but which can be far away in the nucleus (Fig. S3). Instead we propose using the geodesic distance. The length of the shortest path not crossing the cell wall is considered to be the geodesic distance between A and B. The geodesic distance was calculated using the fast marching toolbox from Peyre (28)

A second adjustment concerns the location of the maximal displacement: We assume that the displacement is maximal along the contour of the mapping. Hence, to avoid the maximum displacement from falling inside the nucleus, contour points were added at a short distance outside of the nucleus, causing the derivatives of the mapping normal to the contour to equal zero (Fig S4).

IV. Telomere Tracking

In order to study telomere dynamics over time, detected telomeres in consecutive time frames are matched using the Iterative Closes Point method (ICP). This results in a matching where each telomere is matched with exactly one telomere in the next frame. ICP is a greedy algorithm, i.e. the closest telomere points are matched first and this decision is not revised in the remaining of the algorithm. This is iteratively repeated until all telomeres are matched. Manual annotation of tracks and images was performed in ImageJ freeware (http://imagej.nih.gov/ij/) expanded with the MTrackJ plugin (by E.Meijering, http://www.imagescience.org/meijering/).

Results

First, the mapping algorithm was validated on an artificial sequence using a Monte Carlo simulation. The nucleus was approximated by a flattened ellipsoid (80x50x40voxels) in which 60 random points were generated, representing telomere positions. Then the ellipsoid was deformed using a random affine transformation composed of 3 separate components: the three axes of the ellipsoid were independently rescaled (between a factor 0.9 and 1.1), the ellipsoid was rotated (between -5° and +5° along the long axis of the lateral plane and between -10° and +10° along the axial axis) and translated (between -10 and +10 pixels in the lateral direction). 10000 simulations were performed resulting in average telomere displacement of 8.7±15.7 pixels. Subsequent contour matching and point tracking resulted on average (all transformations combined) in a remaining telomere motion of 2.4±3.5 pixels (or 72.5% reduction), only coming from the motion and deformation of the nucleus. This Monte Carlo simulation was repeated with only transformations in the lateral plane, i.e. without the rotation around the ellipsoids axis and without the scaling in the non-lateral direction. The simulation of only lateral transformations resulted in an average remaining telomere motion of 2.2±3.7 pixels (74% reduction).

As it is not known beforehand what the actual mobility of objects in a nucleus will be, it is difficult to validate the algorithm on a real dataset per se. To test the accuracy of the algorithm we diverted to a dataset with maximal resemblance to the natural situation: ECV-TRF2 cells were acquired during fixation with methanol (Fig. 2). From the moment methanol is added, all active and intrinsic motion is blocked. At the same time, the cold methanol induces a contraction of the entire cell, including the nucleus, which persists for about 10 to 20 min. In this setting, telomeres only become displaced by the deformation of the nucleus and the telomeres' centres can therefore serve as ground truth for validation, i.e. the performance of the algorithm can be judged by its' relative minimization of the displacement

error. Although the induced shrinking is relatively isotropic, not all nuclei deform in the same way, not all parts of the nucleus contract equally and not all telomeres relocate equally far. 97 telomeres coming from 3 different nuclei were manually tracked during 7 frames. This resulted in 582 ground true telomere matches. The influence of the different signatures as well as the different radial basis functions used by the mapping was tested. These tests were performed using 60 sample points for the contours; more samples did not yield better results (Fig. S5).

The proposed mapping was compared with a rigid mapping and with a non-rigid mapping based on the Demons registration algorithm (27). The following metrics were used for error estimation: remaining telomere motion, standard deviation of the motion and the number of false matches in the tracking algorithm (Table 1). A linear basis, i.e. polyharmonic basis of degree 1, performed best for all four signatures. From the four signatures tested, the "tangent direction signature" gave the best result.

Finally, the algorithm was applied to a real dataset from transiently transfected $LMNA^{-/-}$ cells. Nuclei from these cells display dramatic, apparently random deformations, which clearly drive a subset of telomeres in a given direction. This makes it difficult to estimate the inherent mobility of the telomeres within the nucleus. 34 telomeres coming from 3 different nuclei where manually tracked over 9 frames. This results in ground truth of 272 telomere displacements. In Fig. 3g an example of the mapping is shown. As can be seen, the mapping removes most of the motion stemming from nuclear deformation (Table 2). These results show a significant difference over non-processed data. Again, these results confirm the biharmonic radial basis and directional signature as the proposed mapping which results in the least telomere displacement. Note that this displacement is not a measure of error since these results come from living nuclei, i.e. there is still telomere motion coming from other biological processes than nuclear deformation. This telomere motion should however be

considerable smaller than the motion induced due to nuclear deformation and displacement. It took on average 2689 ms to calculate the weighting parameters used by the mapping, i.e. $\mathbf{w}_j$, a, a_x and a_y in eq.(3). For each telomere, the calculation of its mapped location took an additional 103 ms. Approximately 90% of this time was spent on the calculation of the geodesic distance between points. There is no significant difference in computational time depending on the type of signature or on the type of basis function used.

Discussion

In this paper, a new framework was proposed for registering cell nuclei without any shape constraints. The proposed technique works in two steps. First, nuclear boundaries are optimally aligned through time, by matching their contour signatures. Of the four different contour signatures that were tested, the tangent direction signature proved most efficient. Based on this contour matching, the entire nucleus is mapped using polyharmonic interpolation, radial basis of degree one gave the best results.

The primary goal of this registration procedure was to enable an accurate estimation of telomere mobility in living human cells undergoing dramatic nuclear deformations such as human $LMNA^{-/-}$cells. The nuclei in the 4D CLEM recordings display both arbitrary nuclear deformation as well as stochastic lower level telomere mobility, making it difficult to assess the true performance of the algorithm on these datasets alone. On the other hand, it was difficult to simulate such a multifactorial dynamic system in silico, as this would have required explicit knowledge on the expected biological deformations. Therefore, we have limited the possible deformations in the simulation to affine mappings. In an attempt to overcome the limitations of the simulations, we devised a method for controlled deformation, devoid of individual object mobility, with maximal correspondence to the actual biological topic of interest, namely a chemically induced shrinking procedure. To our knowledge, this is the first time such an approach was adopted for mimicking deformation of biological material in an uncompromised and controlled manner. Using this approach, we demonstrated a 5.5 fold increase in localization accuracy with respect to the raw data set. Some part of the remaining error of 64.85nm/min might be due to the contribution of axial components in 3-dimensional movements, which may have been obscured by mapping the deformation in 2D (on projections). However, both cell types under investigation, are adherent and have a flattened nuclear shape in the axial direction suggesting that most of the deformation does

indeed occur in the lateral direction (28). Nevertheless, neither axial deformation, nor rolling phenomena can be excluded. In order to estimate the influence of these non-lateral deformations, simulations with all possible 3-dimensional deformations were compared with simulations containing only lateral deformations. This resulted in a net gain of 2% removal of telomere motion compared to the full deformation simulations (74% vs. 72%), which shows that lateral movements indeed cause most of the motion and 2D mapping already removes a major contribution. This is based on the assumption of an affine transformation, whereas nuclei are obviously not restricted to this type of stringent transformations. Future work should therefore focus on extending the method to 3D, e.g. by warping contours on a slice by slice basis, or by using more complex 3D shape matching techniques, before applying a 3D polyharmonic spline mapping. Next to the error induced by 2D mapping, both the interpolation as the shape matching will introduce a small error as well as both are approximations of the real biological deformation. Nevertheless, despite of these approximations, the proposed method performs better than the current state of the art (27).

The essential novelty of our work is that, in contrast to most of the existing methods, no specific shape constraints are imposed on the contour mapping (10, 12). By doing so, it is possible to map contours with a non-elliptical shape, which lack clear and stable landmarks. Some work has been done on generic non-rigid registration without shape constraints (13,14,27), but several of these methods use intensity, which is unstable and highly variable in fluorescent images (27). Yang et al. proposed a method based on the mapping of segmented nuclei, but their method has difficulties coping with strong deformations, e.g. not all telomeres are mapped inside the nucleus (27). The only work known to the authors handling strongly deformed nuclei restricts itself to shape analysis (29), but does not result in a nuclear mapping, which enables the study of telomere dynamics.

In live cell data it became clear that this type of registration is essential for accurate

estimation of the telomere displacements. The average displacement was reduced by a factor 2.8 with the proposed mapping: 189±42 nm/min without registration, and 66±11 nm/min using the mapping. This obviously emphasizes the importance of the mapping for accurate mobility estimations. The mapping does not only influence the quantitative measurements of telomere dynamics, but also has a positive impact on the tracking algorithm. The number of false matches in the iterative closest point algorithm reduces with approximately 61%, which is an improvement of 12% compared to registration using the Demons method (27).

In conclusion, we have presented a novel registration technique for mapping strongly deformable nuclei. Given to the large variety of pathologies (laminopathies, cancer…) and processes (apoptosis, mitosis) that are associated with strong nuclear deformations, it can be expected that this algorithm will have generic value and improve the accuracy of subnuclear mobility of genome loci as well as nuclear protein bodies.

Appendix

A wide variety of signatures to describe shapes have been proposed in literature (23,24). Within this work, the following four different signatures were tested:

- A complex signature: $s(t) = x(t) + jy(t)$, where $j^2 = -1$.
- a normalized centroid distance signature: $s(t) = \frac{\sqrt{(x(t)-x_c)^2 + (y(t)-y_c)^2}}{\sum_{i=1}^{N}\sqrt{(x(i)-x_c)^2 + (y(i)-y_c)^2}}$,

  where (x_c, y_c) is the centroid of $\mathbf{c}$. Note that this signature is normalized such that $\int s(t)dt = 1$. By doing so, this signature is scaling invariant, i.e. invariant to istropic expansion and contraction of the nucleus.
- A direction signature: $s(t) = \cos^{-1}\left(\frac{y(t+1) - y(t-1)}{\|\mathbf{c}(t+1) - \mathbf{c}(t-1)\|}\right)$. This signature corresponds to the orientation of the tangent line at point (t).
- A curvature signature: $s(t) = \left\|\frac{\partial^2 \mathbf{c}(t)}{\partial t^2}\right\|$. The curvature signature has low values at places where the contour is smooth and high values at places where the contour has sharp angles.

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Figure Legends

Figure 1. The workflow of the proposed method: (a) The nucleus is first segmented and smooth outlines are generated (red) (b) A 1-dimensional contour signature is calculated for the segmented nucleus (c) The signatures of two consecutive frames are matched according to the dynamic time warping technique (d) The alignment matrix, $D(.,.)$, between a signal and a cyclic shifted version of the same signal is shown. The optimal warping path is represented by gray solid squares. The mismatch of the starting point corresponds with the horizontal part at the beginning of the warping path (e) The signatures are resampled such that there is a point-to-point correspondence (f) Based on the contour matching, a full nucleus mapping is calculated using polyharmonic spline interpolation.

Figure 2. Contour mapping applied to a chemically shrunken nucleus. ECV-TRF2 cells were acquired at 30sec intervals with in fluxo addition of methanol after 5min of acquisition (a-c) Fluorescent micrographs showing progressive shrinking throughout the 25min acquisition period (d) Telomere motion over a period of 15 minutes, represented as color-coded tracks of the telomeric centres of mass. The calibration ranges from 10min (white) to 25min (dark blue) (e) Deformation of the nucleus over the same period, represented by the nuclear outlines of consecutive time points (f) The nuclear mapping result after contour matching using a complex signature and a radial basis of degree 1, superimposed onto the outlines of the nucleus at 10min (red) and 25min (blue). The gradient displacement calibration bar ranges from 28nm (black) to 2990nm (white) (g) The result of telomere mapping after contour matching using a complex signature and a radial basis of degree 1. The red and blue curves are the contours of the nucleus at 10min and 25min and the red and blue dots mark the centroids of the telomeres of the respective time points. The green dots represent the location

of the telomeres predicted by the mapping, i.e. solely based on the deformation.

Figure 3. Contour mapping applied on naturally deforming nucleus from an LMNA$^{-/-}$ cell, transiently transfected with pmCitrine-hTRF2, acquired at 2min intervals (a-c) Fluorescent micrographs at different time points. (d) Telomere motion over a period of 18 minutes, represented as color-coded tracks of the telomeric centres of mass (e) Deformation of the nucleus over the same period, represented by the nuclear outlines of consecutive time points (f) The nuclear mapping result after contour matching using a complex signature and a radial basis of degree 1, superimposed onto the outlines of the nucleus at 10min (red) and 25min (blue). The gradient displacement calibration bar ranges from 259nm (black) to 2385nm (white) (g) The result of telomere mapping after contour matching using a complex signature and a radial basis of degree 1. Red, blue and green represent features of the first time point, last time point (18min) and mapping-based prediction, respectively.

Supplementary Material

Movie S1. Composite movie illustrating dramatic nuclear deformations in $LMNA^{-/-}$ cells. Three nuclei of $LMNA^{-/-}$ cells, which were transiently transfected with pmCitrine-hTRF2 and acquired with CLEM at 2min intervals, were stitched together in a single montage. The individual nuclear outlines were derived from the nuclear background signal and superimposed on the time stacks for highlighting the deformation. The scale bar represents 10µm.

Figure S1: The consecutive steps of the segmentation algorithm: (a) low pass filtering of the image, (b) Otsu thresholding, (c) morphological closing, (d) morphological opening (e) low-pass filtering the contour.

Figure S2: Four different shape signatures corresponding with the contour of the nucleus in figure S1 (a) complex signature; (b) normalized centroid distance signature; (c) tangent direction signature and (d) curvature signature

Figure S3: An example of two different distance functions: the Euclidean distance on the left, the geodesic distance on the right. The distance between points A and B corresponds to the length of the dashed line, where the blue line represents the nucleus contour.

Figure S4: Two examples of polyharmonic spline interpolation for nucleus mapping. In (a,c) the gray value at a point in the nucleus corresponds to the magnitude of the displacement vector at that point. (b,d) Is a profile corresponding with the green line in respectively (a) and (c). The top figures correspond to polyharmonic spline mapping with radial basis of degree

two. Note that the maximal displacement of the nucleus doesn't lie on the contour, which is unlikely under the assumption that the interior displacement is due to a deformation of the nuclear contour. The lower figures represent our proposed mapping where the displacement is imposed to be maximal at the contour, also with radial basis of degree 2.

Figure S5: The influence of the number of contour samples on the accuracy of the telomere mapping. Calculated on the methanol dataset, using the tangent direction signature and the polyharmonic basis of degree 1.